THE FUNCTION OF EGGLESS CAPSULES AND LEAF IN NESTS OF THE FROG *PHYLLOMEDUSA HYPOCHONDRIALIS* (ANURA: HYLIDAE)

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Abstract.—Phyllomedusa hypochondrialis makes a purse-like nest over water by folding a leaf around its egg mass. The egg mass contains eggs (embryos) and fluid-filled, eggless capsules. The enclosing leaf and eggless capsules prevent drying and death of embryos during the 8–9 day, nonaquatic, prehatch period. A long prehatch period allows the embryos to attain a relatively advanced developmental stage before entering the water, where they are exposed to many aquatic predators.

Differences in mating characteristics between species in the genera *Phyllomedusa* and *Agalychnis* are due to different physiological and behavioral mechanisms that insure a continuous water supply to the prehatch embryos.

Mating, oviposition, embryonic development and leaf nest formation of Phyllomedusa hypochondrialis were observed by Budgett (1899) in the Paraguayan Chaco. Budgett's work was extended by Pyburn and Glidewell (1971) and Pyburn (1976), who observed populations of P. hypochondrialis on the western llanos of Colombia. Males of P. hypochondrialis begin calling from grasses and shrubs around pools that form during the first heavy rains of the rainy season. With few exceptions only gravid females come to the pools where males are calling. An egg-laden female approaches a calling male, the pair enters into amplexus and the female lays one egg mass on a leaf over water (Fig. 1). The first eggs emerge onto the upper surface of the leaf near its distal end. As laying continues the pair moves upward toward the petiole and both members of the pair, using their hind limbs, fold the leaf around the eggs, making a purse-like nest. After laying, the female disengages herself from the male and immediately leaves the breeding site. According to Budgett (op. cit.) spawning is sometimes continued on another leaf, but I have been unable to confirm this.

The leaf-nest contains about 80 eggs. Each egg is surrounded by its vitelline membrane and, outside of the membrane, by a thick, gelatinous capsule. Distributed among and over the surface of the egg-containing capsules are about 300 fluid-filled, eggless capsules. The fluid in the eggless capsules is probably metabolic water, produced in the body wherever oxidative processes take place (Thorson, 1955). As the eggs (embryos) develop, the space between embryo and vitelline membrane increases and fills with fluid. At the same time the fluid in the eggless capsules diminishes. Before hatching, the walls of the embryonated capsules become thin and tightly stretched. At hatching, the embryo, now a larva, breaks out of its capsule and usually falls into the pool below. If the larva falls on the ground it is capable of flipping into the water with its muscular tail.

Agar (1910) observed that during the development of *Phyllomedusa sau*vagii, a species whose nests are similar to those of *P. hypochondrialis*, the volume of vitelline fluid increased, causing the capsules containing the embryos to swell to twice their original size. Agar stated that the eggless capsules provided fluid for the developing embryos and observed that eggs near the surface of the mass died if they were not covered by the nest leaves.

My objective in the following observations and experiments was to test the hypothesis that the enclosing leaf and eggless capsules of *Phyllomedusa hypochondrialis* nests are necessary to the survival of the embryos during their nonaquatic developmental period.

Materials and Methods

The South American llanos is a vast savanna that extends from the Orinoco River southwesterly, in Colombia, to near the base of the eastern Andes. Its approximate southern boundary is marked by the Guaviare River (Bates, 1948), which, with its tributaries, flows northeasterly into the Orinoco. South of the Guaviare the drainage is southeasterly into the Amazon, and the vegetation is predominantly rain forest. In the Colombian llanos, the grass-covered uplands are broken by islands of close-growing trees known locally as "mata montes." In the humid lowlands broad-leaved trees and mauritia palms form isolated woodlands around lakes and extensive gallery forests along the streams (Beard, 1953).

The work reported herein was done in June and July, 1974, and in April and May, 1976, in and near the villages of Lomalinda and Puerto Lleras. These villages lie near the Ariari River in the southwestern part of the Colombian llanos. Although subject to sudden heavy rains and short dry intervals during the rainy season, the climate of the southwestern llanos is nevertheless regularly cyclic and predictable from year to year. The rainy season begins in early April and lasts 8 months. The average annual rainfall (1975–1977) at Lomalinda is 305 cm (J. K. Salser, personal communication), almost all of which falls between April and November. In the 4-month dry

Fig. 1. Mating sequence in *Phyllomedusa hypochondrialis*. Upper right, calling male; upper left and center, gravid female and calling male approaching each other; lower left, amplectant pair ovipositing in leaf; lower right, separation of pair after laying.



season very little rain falls and there may be long periods, 50 days or more, with no rain at all.

I observed developing egg clutches under both natural and artificial conditions. In either case I attempted to disturb the embryos as little as possible except for the variable being tested. Details of methods of handling differed in different circumstances and are stated in the appropriate places below. Temperature readings were made to the nearest 0.1 C with a Schultheis, narrow bulb quick-stabilizing thermometer. Developmental stages are those of Pyburn (1963) as modified from Gosner (1960). Egg nests used in indoor experiments were held in open plastic pans on a table about 75 cm above the floor and were not exposed to the direct sun. The room was ventilated by windows in its eastern and western walls, but the room temperature was not constant. The maximum recorded room temperature was 28.1 C; maximum recorded outside shade temperature was 43.0 C.

Experiments with Naturally Occurring Nests

I studied naturally occurring nests of *Phyllomedusa hypochondrialis* at several grassy pools at Lomalinda and nearby Finca Bon Aire. The pools were partly lined with low shrubs, and supported dense stands of emergent cane-like grass. Other frogs breeding at these pools at this time were *Hyla blairi*, *H. crepitans*, *H. leucophyllata*, *H. punctata*, *H. rostrata*, *H. wandae*, *Phrynohyas venulosa*, and *Pseudis paradoxus*. In addition to the larvae of these species the pools supported populations of dragonfly naiads, belostomatid bugs, ditiscid beetles (all of which prey on anuran larvae) and many other invertebrates.

The *P. hypochondrialis* enclosed their eggs in leaves of the emergent grass and in shrub leaves overhanging the pools from a few cm to about 1 m above the water. In order to test the effect of the enclosing leaf on embryo survival under natural conditions, I exposed the embryos of 6 nests at one pool by folding the leaf back to its original flat shape. Otherwise, I left each leaf in place with its egg mass undisturbed and firmly fixed to the leaf surface. Six nearby egg nests were noted but left unopened to serve as partial controls. I tried opening and resealing several nests but found that, once opened, the leaves would not stay closed of their own accord. Eggs of the exposed clutches were too compactly fused to each other and to the leaf to permit counting at the beginning of the experiment without weakening their attachment. Mortality figures for the experimental clutches were therefore based upon estimates of the number of dead or missing embryos in each clutch relative to the average number of embryos in the control clutches.

The 6 experimental clutches were exposed at about 2200 h, 14 April and were examined at 24 h intervals on the following 3 nights. A heavy rain fell about midnight on the 14th, after the leaves had been opened. No rain fell

on the 15th and none fell during the day on the 16th. Both days were hot; at Lomalinda the air temperature rose to 43 C by noon on the 16th. A hard rain fell the night of the 16th.

Examination of the 6 egg nests on the next evening after they were opened revealed that 4 were developing normally, that most of another clutch was developing normally but about 30% of its embryos were missing, and that one entire clutch was missing from its leaf. Loss of embryos of the latter 2 clutches was most likely due to predation. By the evening of the 17th all the embryos in another clutch were dead and dried and the 4 remaining clutches all contained some dead and dried embryos. In all clutches the eggless capsules had collapsed and dried and the fluid of the embryonated capsules (perivitelline fluid) had darkened. Estimated mortality in the 6 experimental clutches was 100%, 100%, 5%, 40%, 65%, and 72%.

The 6 control clutches showed no evidence of predation or mechanical disturbance. When opened after the termination of the experiment, two clutches were developing normally and 4 contained dead embryos in the following percentages: 11.8%, 1.9%, 7.1%, and 1.3%. The perivitelline fluid of the live embryos had darkened. The mean number of eggs in the control clutches was 80.0 (range, 66 to 105). In all control clutches the mortality was 3.8%. Mortality in the 6 experimental clutches, based on an average of 80 eggs per clutch, was 65.8%.

Outdoor Experiments under Artificial Conditions

In order to test further the possible protective function of the enclosing leaf I attached 6 leaf nests to the vertical side of a western facing wooden, dark brown wall at 1200 h, 2 May 1976. The leaves were attached 1.5 m above the ground, in pairs, 15 cm between pairs and 2 cm between members of a pair. Eaves sheltered the clutches from the midday sun but not from the afternoon and evening sun, nor from rain coming from the west. The leaf of one member of each pair was opened, the other opened and reclosed. Each leaf was attached to the wall by a small brad. The condition of the eggs on the opened leaves was noted 8 times at irregular intervals over the next 3 days. During the day the weather was sunny to partly cloudy and warm. Rain fell irregularly in the daytime and every night. The highest air temperature recorded was 30.3 C at 1540 h on 2 May. At noon, 2 May, temperature readings of 2 clutch pairs were: 1st pair-air midway between clutches, 26.8 C; jelly of open clutch, 24.8 C; jelly of closed clutch, 26.4 C; 2nd pair-air midway between clutches, 28.5 C; jelly of open clutch, 26.4 C; jelly of closed clutch, 27.4 C. These data indicate a greater rate of evaporative water loss from the open clutches than from the closed clutches.

Evidence of shrinkage and excessive darkening (yellowing) of the capsular fluids was first noted in the three open clutches about 1530 h on 2 May.



Later that evening the clutches were wet by rain and all had recovered from the shrinkage of the previous day by 0645 h, 3 May. By 1115 h, 3 May, shrinking, drying, and yellowing were again in evidence and this trend continued until all of the embryos on the open leaves died. The eggless capsules became completely dry and the embryonated capsules shrank and became yellowish amber brown. By 1030 h, 4 May the embryos had shrunk to tiny amber-like beads and all were dead.

Among the 3 unexposed clutches, one nest had one dead embryo at the upper end of the clutch where it had not been covered by the leaf and five live embryos had shrunken capsules. About half of the eggless capsules in each nest had lost water and collapsed. Otherwise all of the embryos were developing normally when the leaves were opened at 1030 h, 4 May.

Indoor Experiments

To test the hypothesis that the eggless capsules contribute necessary water to the embryos (Fig. 3), I divided a clutch of *P. hypochondrialis* embryos (stage 18) into three lots as follows: 32 embryonated capsules, lot A, were transferred from the original leaf to another leaf of the same plant (*Lantana* sp.) and the leaf was folded around the embryos in the manner of a naturally formed nest. The edges of the leaf were fastened together with an insect pin. About 150 eggless capsules were included in this lot. A group of 21 embryonated capsules (lot B) was treated in the same way except that no eggless capsules were included with this lot. Neither of the leaves containing lots A and B was moistened during the experiment. The remaining 10 embryonated capsules (lot C) were submerged, without eggless capsules, in pond water. The lots were inspected irregularly and any dead embryos were counted and removed.

The submerged embryos (lot C) did not die immediately, contrary to the statement of Budgett (1899), but none advanced beyond stage 19 (heartbeat) and all died within 52 h after submergence.

The embryos of lot B began dying after 25 h (Fig. 4) although the developmental rate of the survivors did not fall behind the rate of the controls (lot A) until about 75 h from the beginning of the experiment. Mortality in lot B accelerated after 68 h and none survived beyond 99 h. Death of embryos began at either end of the mass, where the leaf was open, and proceeded inward, those embryos at the center being the last to die. Deaths

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Fig. 2. Mating sequence in *Agalychnis callidryas*. Upper left and right, calling male approached by gravid female; lower left, amplectant pair descending to pool; lower right, pair in pool taking up water; middle right, pair ascending; lower middle, pair ovipositing on leaf.



Fig. 3. Test of hypothesis that eggless capsules (small circles) contribute essential water to capsuled embryos (large circles) during terrestrial development. Numbers in parenthesis indicate sequence of experiments and letters designate egg lots as discussed in the text.



Fig. 4. Mortality of embryos of *Phyllomedusa hypochondrialis* in the absence of eggless capsules. See Fig. 3 (1).

were always preceded by reduction in capsular volume. Ninety-four per cent of the controls (lot A) survived beyond 99 h. As the leaf dried the eggless capsules adhered closely to the surface of the embryonated capsules and became smaller as the latter enlarged.

I repeated the experiment, with certain modifications, and obtained similar results (Fig. 5). In this second experiment two groups of 23 (lot A) and 25 (lot B) embryonated capsules were separated from each other but were wrapped in the original leaf. Lot A included about 100 eggless capsules, lot B none. A third group (lot C) of 21 embryonated capsules and about 100 eggless capsules was submerged in pond water. All embryos were from the same clutch and all were in stage 20 (gill circulation) at the beginning of the experiment. The submerged embryos advanced very little, none past late stage 20, and all eventually died. Most of the embryos of lot B developed normally during the first 20 hours then died between 20 and 32 hours after the beginning of the experiment. During the latter interval the capsules lost volume, which greatly restricted the movement of the embryos. The embryos of lot A (controls) developed normally and all survived beyond 32 hours. In the latter part of the experiment the volume of the eggless capsules decreased and some adhered closely to the surface of the embryonated capsules.



Fig. 5. Mortality of embryos of *Phyllomedusa hypochondrialis* in absence of eggless capsules. See Fig. 3 (2).

In a third experiment (Fig. 6) I divided a newly deposited egg mass into two groups of 32 (lot A) and 28 (lot B) encapsulated embryos. About 100 eggless capsules were included with lot A and none with lot B. The two lots were wrapped in separate leaves and the leaves wrapped in moist paper towels. Both lots were kept moist with pond water throughout the experiment. After about 12 hours, 10 embryos in lot A were destroyed by a cockroach (*Periplaneta* sp.), and toward the end of the experiment four eggs in lot B died from unknown causes. Otherwise all embryos developed normally and hatched after eight days.

In all of these experiments the capsular fluid of both experimental and control groups darkened as the embryos aged.

Results

The embryos of *Phyllomedusa hypochondrialis* usually died, even in the presence of eggless capsules, if exposed to the normal conditions of their habitat by opening the leaf cover. However, rain delayed or prevented the death of exposed embryos. The enclosing leaf of unopened nests protected the embryos against drying and death.

The leaf did not prevent the death of embryos in the absence of eggless capsules, unless the embryos received water from some other source. The



Fig. 6. Survival of embryos of *Phyllomedusa hypochondrialis* in the absence of eggless capsules when kept moist. See Fig. 3 (3).

embryos survived if eggless capsules were left with the clutch or, in the absence of eggless capsules, if the clutch was artificially moistened with pond water.

The results of the experiments and observations support Agar's (1910) statement that the eggless capsules supply water to the developing embryos. Furthermore, the results show that both leaf and eggless capsules are necessary for maximum survival of embryos during the nonaquatic period of development.

Discussion

Phyllomedusa hypochondrialis, like other phyllomedusine frogs (Agar, 1910; Kenny, 1966; Lescure, 1975; Lutz and Lutz, 1939; Oliver, 1937; Pyburn, 1970; Salthe and Duellman, 1973; Wiewandt, 1971), lays relatively small clutches of large-yolked eggs that have a long prehatch developmental period. Hence phyllomedusines may be said to have K-selected life history strategies in the sense of MacArthur and Wilson (1967:149), relative to frogs that lay large numbers of small-yolked eggs directly in water (see Crump, 1974). Eggs of frogs in the latter group often hatch as helpless embryos in stages 16–18 after only 24–28 h of development (Limbaugh and Volpe, 1957; Pyburn, 1967; Zweifel, 1964), whereas *P. hypochondrialis* eggs undergo 8–9 days of prehatch development (Pyburn, 1976) and upon hatching enter the water as advanced (stage 23), highly mobile larvae. As a result of their advanced condition at hatching phyllomedusines are immediately capable

of detecting and escaping the many aquatic predators that commonly attack tadpoles (see Herreid and Kinney, 1966; Young, 1967; Brockelman, 1969; Heyer *et al.* 1975; McDiarmid and Heyer, 1974). The long prehatch period of *P. hypochondrialis* is possible not only because of each embryo's large yolk supply, but also because the enclosing leaf and water-storing capsules prevent drying and death during the terrestrial period. The leaf-nest of *P. hypochondrialis* is functionally analagous to an amniote egg.

The use of water from the eggless capsules by the embryos of *Phyllo-medusa* can be explained from studies of species whose embryos normally develop in pond water and from well-known principles governing the movement of materials in solution. In *Rana*, shortly after fertilization, the ovum shrinks away from the vitelline membrane (Løvtrup, 1962) and secretes corticle granules containing polysaccharides into the perivitelline space (Konopacki, 1924; Salthe, 1965). The membrane is apparently impermeable to the polysaccharides which therefore exert an osmotic pressure causing water intake from the pond into the perivitelline space. As development continues proteins are secreted into the perivitelline fluid along with metabolic products of low molecular weight, including ammonia and urea. The membrane is permeable to these metabolites, which are continuously secreted at an increasing rate (Salthe, 1965). Ammonia and urea are toxic in high concentrations, but because they diffuse readily through the vitelline membrane they are not hazardous to embryos developing in pond water.

To nonaquatic embryos like P. hypochondrialis, that must rely on a limited external water source (the eggless capsules), the accumulation of nitrogenous wastes is a potential danger. In early developmental stages the increasing osmotic pressure of the perivitelline fluid causes water to diffuse from the adjacent eggless capsules into the fluid around the embryo, diluting the fluid and preventing the waste concentration from reaching a lethal level. Furthermore, there is initially a net movement of ammonia and urea from the perivitelline fluid into the fluid of the eggless capsules. However, because of the presence of proteins and polysaccharides, the initial loss of metabolites does not effectively lower the osmotic pressure of the perivitelline fluid, into which water continues to diffuse from the eggless capsules. The resulting increase in volume enclosed by the embryo's capsule and vitelline membrane permits free movement of the embryo's elongating tail and branching gills, while the capsule wall and membrane become thinly stretched. Because of the limited amount of water stored in the eggless capsules, a point is eventually reached at which the rate of dilution of waste can no longer keep pace with the increasing waste concentration in the perivitelline fluid. Excessive accumulation of metabolites in late prehatch stages is evidenced by the darkening amber color of the perivitelline fluid. At this point the embryo hatches. Thinning and eventual rupture of the capsular wall (hatching) may be due to the action of hatching enzymes

(Kobayashi, 1954), but rupture of the capsule seems to be aided by tail movements of the embryo, which increase in vigor as metabolite concentration increases.

Supplying the embryos with a large yolk reserve and stored water, and enclosing them in a shell-like leaf are all forms of parental care that require expenditure of reproductive energy, but at the same time permit a long prehatch developmental period that enhances the probability of survival of each hatchling after it enters the water.

Probably all phyllomedusines lay their eggs out of water and have prolonged prehatch periods, but strategies for delayed hatching differ among the species. Members of the genus Agalychnis (callidryas, dacnicolor, moreleti) oviposit on open leaves (Lutz, 1950) and do not supply the clutch with eggless capsules. Instead, the amplectant frogs go into the pool before laying, where they take up water to be released later as the eggs are deposited on a leaf (Fig. 2; Pyburn, 1970). The released water is stored in the copious jelly around the eggs and gradually diffuses into the perivitelline fluid as the osmotic pressure of the fluid increases. Agalychnis callidryas and A. moreleti sometimes oviposit on leaves that are several m above water (Fouquette, 1968; Duellmann, 1970:115). Nevertheless, before depositing each egg mass the mated pair goes back into the pool to replenish its water supply. These long trips between pool and oviposition site, in addition to increasing the time of exposure to predation, necessitate an energy expenditure by Agalychnis that could be spent otherwise if pond water were not required for embryonic development.

Unlike Agalychnis, Phyllomedusa hypochondrialis does not take water from the pool while in amplexus, and therefore is not as long exposed to predation during the egg-laying process. However, as in other members of the genus Phyllomedusa (Crump, 1974), P. hypochondrialis must allocate energy for nest construction and the production of the fluid-containing capsules, which are probably formed in the oviduct (Agar, 1910). Thus differences in mating characteristics between species in the genera Agalychnis and Phyllomedusa are due to different physiological and behavioral mechanisms for insuring a continuous supply of water to the prehatch embryos.

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